IMMUNOHISTOCHEMICAL ANALYSIS OF EXPRESSION OF CD 15 IN VARIOUS THYROID NEOPLASMS

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CERTIFICATE

CERTIFICATE

This is to certify that the dissertation work entitled "**IMMUNOHISTOCHEMICAL ANALYSIS OF EXPRESSION OF CD 15 IN VARIOUS THYROID NEOPLASMS"** submitted by Dr. K.M. Rajeshwari is work done by her during the period of study in the department of Pathology, PSG IMS & R from June 2008 to February 2011. This work was done under the guidance of Dr. S.Shanthakumari, Professor, Department of Pathology.

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INTRODUCTION

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The thyroid gland is one of the most responsive organs in the body and is a large store of hormones. The gland responds to many stimuli and is in a constant state of adaptation. Thyroid hormones regulate the body's metabolism too. They have a bearing on the heart rate, cholesterol level, body weight, energy level, muscle strength, skin condition, menstrual regularity, memory function and many other conditions [1]. Diseases of thyroid gland especially mass lesions are easily accessible and amenable to surgical treatment and cure.

Thyroid malignancies represent the most common malignancies of the endocrine system. With environmental and genetic factors playing a role, there is an increase in incidence of these malignancies. Due to the dependence of thyroid on environmental iodine, it is particularly vulnerable to the genotoxic effects (DNA damage) of radioactive iodine and to the non genotoxic effects (TSH stimulation) resulting from iodine deficiency. The role of iodine is mediated through the growth stimulatory effects of high TSH levels [2].

With the use of improved and sophisticated diagnostic tools like guided Fine needle aspiration, PET scan etc., the incidence of detecting early thyroid neoplasms are on the rise, which pose challenge to the surgeons and to the pathologists.

The challenges for prognostication of these tumors are confounded as these neoplasms behave in an indolent fashion in many and aggressive in a few. Specific clinical and pathologic criteria are utilized to predict the biological behaviour of tumors as an achievement in modern medicine. Staging criteria are used to evaluate the tumor not only for predicting prognosis of these tumors, but also to select appropriate therapy. The current staging parameters do not help in predicting patients who are likely to have a relapse. Hence a lot of molecules are being studied which would not only help to predict the biological behaviour, but also to plan scientific basis for therapy. One such molecule of interest is CD15.

CD15 (Leu M1, Cluster differentiation antigen 15) a blood group antigen, is a complex cluster of cell surface glycoproteins and glycolipids having a common terminal pentasaccaride known as Lewis X (Le^x) antigen. Lewis X is a fucosylated derivative of N-acetyl lactosamine, a cell surface glycoprotein [3].

Synthesis of blood group antigens in the thyroid tissue is considered as 'oncofetal', as they are not detected in the normal adult organ. Carcinomas of the thyroid gland show a significant increase in expression of these antigens possibly secondary to oncogenic transformation and subsequent increased production of Lewis X molecules (Lacto-N-fucopentaose III ceramide), a component of the CD15 complex [4].

Recent studies have shown the utility of CD15 as a prognostic marker. There are no studies from India which have looked into the expression of this molecule in thyroid neoplasms. This study therefore attempts to identify the expression of CD15 on the various thyroid neoplasms encountered in the study period.

AIMS AND OBJECTIVES

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The aims of this study are

1. To study the incidence of Immunohistochemical expression of CD-15 in various thyroid neoplasms

2. To correlate the expression of CD15 with clinical presentation and stage of the tumor.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The thyroid gland develops from two lateral anlages and a median anlage. The medial anlage develops from the base of tongue, in the foramen cecum and becomes bilobed forming the greater portion of the thyroid gland and gives rise to the follicular epithelial cells. The follicular cells utilize iodine to form thyroxine and triiodothyronine. The lateral anlage develops as ultimobranchial bodies from the endoderm of fourth and fifth branchial pouches [4]. The ultimobranchial body fuses with the median anlage giving rise to the parafollicular C cells which secretes calcitonin.

Thyroid neoplasms account for approximately 1% of all cancer cases in the developed countries [1]. It affects all age groups although they are rare in children. The incidence increases throughout life. A female preponderance has been noted among patients in early and mid adult life, perhaps related to the expression of estrogen receptors on the tumor cells [5]. In contrast, neoplasms in childhood and late adult life are equally distributed with no sex predilection. It has been shown that thyroid nodules in older males harbor malignancy more commonly than in females with similar lesions.

Thyroid cancer is the only malignancy with age as one of the major prognostic indicator in the majority of staging systems. The mortality rate with thyroid cancer climbs gradually starting at age 40–45. With the same degree of cancer involvement, patients aged less than 45 have a distinctly different

prognosis than those aged more than 45 years. The reason for the distinctly different prognosis based on age is not entirely clear. The fact that the relationship exists suggests that there is something intrinsic to either the cancer or the treatment that is age dependent [6]. The association between advanced age and poor prognosis with thyroid cancer was hypothesized to a decline in immune system with age and a general increase in mortality from all causes with age [6].

The incidence of thyroid neoplasms depends on the degree of iodine deficiency in the area. The ratio of papillary carcinoma to follicular carcinoma is higher in areas not endemic for iodine deficiency. Anaplastic carcinoma and follicular carcinoma are more often seen in iodine deficiency areas. Radiation is more closely associated with the development of papillary carcinoma than follicular neoplasms by causing double stand breaks in DNA, a precursor for RET and TRK rearrangements [7].

A Chennai population based carcinoma registry released in 1984 showed that thyroid neoplasms constituted for about 1% and 2% of all incident cancers among males and females respectively. Cumulative lifetime risk of thyroid cancer in Chennai was one in 970 for males and one in 565 for females [8]. A high dietary intake of iodine was proposed as a risk factor for the development of papillary carcinoma [9].

Table 1 gives a comparison of incidences of major types of malignant thyroid tumors between the reported world literature and that observed by Rosai J et al, Department of Pathology, SGPGIMS, Lucknow, India [10].

TABLE I:

Type of Tumor	**World Literature**	**SGPGIMS, Lucknow, India**
Papillary Carcinoma	60-80%	64 %
Follicular Carcinoma	5-25 %	20%
Medullary Carcinoma	5-10 %	7%
Poorly differentiated (Insular) Carcinoma	1-10 %	5%
Undifferentiated (Anaplastic) Carcinoma	4-10 %	4%

Primary epithelial neoplasms of thyroid originate from follicular epithelial cells. Follicular cell derived tumors include follicular adenoma, follicular carcinoma, papillary carcinoma and its variants, poorly differentiated carcinoma and anaplastic carcinoma. Medullary carcinoma is a C- cell derived neoplasm comprising less than 10% of all malignant thyroid tumors [4]. Non epithelial tumors are very rare which include lymphoid malignancies and mesenchymal tumors. Metastatic tumors are very rarely encountered in the thyroid [11].

Among epithelial tumors, carcinomas of follicular origin outnumber those of C- cell origin [4]. Tumors arising from follicular epithelial cells exhibit a wide range of biologic behaviour. Papillary carcinoma is the commonest of all thyroid neoplasms. It behaves in an indolent fashion with a very low mortality. Medullary carcinoma and anaplastic carcinoma present as aggressive tumors leading to death within a year of diagnosis. Due to the heterogeneous behaviour of thyroid neoplasms, a need for correct histological diagnosis and classification is essential.

CLASSIFICATION OF THYROID TUMORS (WHO 2004) [2]:

BENIGN

- Follicular adenoma

MALIGNANT

- Papillary carcinoma
- Follicular carcinoma
- Medullary carcinoma
- Poorly differentiated carcinoma (Anaplastic)
- Undifferentiated carcinoma (Insular)
- Mucinous carcinoma
- Muco- epidermoid carcinoma
- Squamous cell carcinoma
- Others

FOLLICULAR ADENOMA:

Follicular adenoma is a benign tumor showing a uniform pattern throughout and is enveloped by a thick fibrous capsule. Morphologically, it is composed of closely packed follicles, trabeculae or solid sheets of cuboidal or low columnar cells with pale or darkly stained nuclei and inconspicuous nucleoli. Prominent hyalinization and vasculature can be seen [9].

Rare variants include atypical follicular adenoma, hyalinizing trabecular adenoma and Signet- ring cell follicular adenoma. Atypical follicular adenoma shows increased cellularity, mitoses, spontaneous necrosis or infarction and lack invasive growth [4, 9]. Hyalinizing trabecular adenoma is characterized by arrangement of the elongated tumor cells in a wavy trabecular pattern around capillaries in a background of lumpy hyaline material and calcified extracellular matrix. Nuclear grooves, pseudoinclusions and peri nuclear haloes are prominent [4]. Signet- ring cell follicular adenoma shows a predominance of signet ring cells with discrete cytoplasmic vacuoles, intermixed with groups of follicular cells of normal cytologic features [4].

Hurthle cell adenomas are considered a subtype of follicular adenoma. They are bright brown macroscopically. The neoplasm is characterized by the presence of Hurthle cells arranged in follicular and / or trabecular pattern with partial or complete encapsulation. Morphologically, the Hurthle cells are large with voluminous eosinophilic cytoplasm and a round nucleus [11]. The granular

appearance of the cytoplasm is due to the accumulation of abundant mitochondria. These cells were first described by Askanazy in 1898 in Graves-Basedow disease. It has a tendency for spontaneous infarction [11].

PAPILLARY CARCINOMA:

Papillary carcinoma is the most common of all primary thyroid malignancies, accounting for about 70- 85% of cases [1]. It can affect any age and has a female preponderance. Papillary carcinoma is characterized by intrathyroidal invasion resulting in multifocal disease and metastasizes to regional lymph nodes. Extra thyroidal extension can occur and is defined grossly by Woolner et al as 'extension of the tumor well beyond the capsule of the thyroid gland to involve such structures as the larynx, trachea or esophagus' [12]. Extra thyroidal extension was defined microscopically by Carcangiu et al in their series as tumor 'intimately admixed with the soft tissues, including skeletal muscle' of the neck [13].

Microscopically, classic type is characterized by formation of complex arborizing papillae with a central fibro vascular core. The papillae are covered by one or more layers of cells with crowded oval nuclei. The nuclei are enlarged and exhibit margination of chromatin, overlapping, with nuclear grooving and intra nuclear cytoplasmic pseudo inclusions [1, 4]. However, papillary thyroid carcinoma can exhibit a pure follicular pattern or mixed papillary and follicular pattern. Psammoma bodies, the lamellated concretions

formed by deposition of calcium upon the dying cells of the papillae are virtually pathognomonic of papillary carcinoma [11].

The relation between papillary carcinoma and chronic lymphocytic thyroiditis is controversial [14, 15]. Reports by Livolsi suggested that the lymphocytic infiltration of the surrounding thyroid tissue is induced through autoimmune mechanisms triggered during the development of papillary carcinoma [14]. Singh et al found an increased prevalence of Hashimoto thyroiditis in patients with papillary thyroid carcinoma. The presence of coexistent Hashimoto thyroiditis did not affect the diagnostic evaluation or management of papillary thyroid cancers. They further hypothesized that the survival may be superior in patients who have papillary thyroid cancers with coexistent Hashimoto thyroiditis [15].

VARIANTS OF PAPILLARY CARCINOMA:

Several morphological variants of papillary carcinoma have been recognized based on the architecture, growth pattern, cellular morphology and stromal features. The recognition of these subtypes becomes important as some of them are more aggressive in their biologic and clinical behaviour irrespective of their bland appearance.

I. Papillary microcarcinoma:

Papillary carcinomas measuring 1 cm or less in diameter are termed as papillary micro carcinomas. The tumor is most often unencapsulated and sclerosing [2]. A familial occurrence has been reported by Lupoli et al in a subset of patients with papillary micro carcinoma [16]. It is frequently detected in autopsy or in thyroidectomy specimens for other indications and is associated with an excellent prognosis despite occasional regional lymph node metastasis.

II. Follicular variant:

Under the microscope, the neoplasm is composed of small to medium sized, irregularly shaped follicles with virtually no papillary structures. Stromal sclerosis and psammoma bodies can be present. Characteristic nuclear features of papillary carcinoma are evident [17]. Distant metastasis and vascular invasion are common. Nodal metastasis and extra thyroidal extension are rare. Some of these tumors exhibit encapsulation with an exceptionally good prognosis (so called Lindsay tumor) [18]. The behaviour is similar to that of conventional papillary carcinoma.

III. Tall cell variant:

This is a rare variant and consists predominantly of tumor cells whose heights are at least three times their widths. Tall cells must be seen in 50% or more of the tumor areas to make a diagnosis of tall cell variant of papillary

thyroid carcinoma. Necrosis, mitotic activity and extra thyroidal extension are common. These tumors occur in older patients, often males, with a more aggressive clinical behaviour [17]. Tall cell variant of papillary carcinoma frequently show strong immunostaining for CD15 expression.

IV. Oncocytic variant:

Oncocytic papillary carcinomas are characterized grossly, by a distinct mahogany appearance. The complex branching papillae have a thin fibro vascular stromal core, covered by a single layer of polygonal oncocytic cells with abundant granular eosinophilic cytoplasm [2, 17].

V. Warthin tumor like variant:

This variant has a brisk lymphoplasmacytic infiltrate in the papillary stalks with circumscription and central cyst formation. Papillae and follicles were lined by oncocytic cells with the typical nuclear features of usual papillary carcinoma. Psammoma bodies can be seen. It is frequently associated with Hashimoto thyroiditis [19, 20]. The immuno profile of the lymphoid cells is no different from chronic lymphocytic thyroiditis.

VI. Cribriform morular variant:

Focal papillary architecture, cribriform features, solid and spindle cell areas interspersed with squamoid morules, characterize this variant of papillary carcinoma. The nuclei frequently harbor eosinophilic, homogenous cytoplasmic

inclusions. It typically occurs in patients with familial adenomatous polyposis or Gardner syndrome [4]. In this setting, the tumor is often multifocal and occurs in young women [17].

VII. Clear cell variant:

Tumor cells have abundant clear or vacuolated cytoplasm. Cytoplasmic clearing is usually due to accumulation of glycogen. The nuclear features are otherwise typical of papillary carcinoma. Recognition of this variant in metastatic sites may be problematic [17, 18].

VIII. Diffuse sclerosing variant:

This is an unusual variant of papillary carcinoma seen more frequently in children and is associated with a poor prognosis. Diffuse involvement of both the lobes is characteristic. The microscopic hallmark is the presence of widespread intra thyroid lymphatic permeation by numerous neoplastic micro papillae and a dense sclerotic stroma [17].

FOLLICULAR CARCINOMA:

It comprises 5-15% of all thyroid malignancies [1]. Follicular carcinoma is more common in women of older age group and in the iodine deficient areas [4]. The tumor can be encapsulated or widely invasive. A true capsular and / or a vascular invasion in a follicular neoplasm [9, 11] is mandatory to diagnose follicular carcinoma.

Capsular invasion is defined by tumor penetration through the tumor capsule unassociated with the site of a previous fine needle aspiration biopsy[21].

Vascular invasion is defined by the presence of intravascular tumor cells either covered with endothelium or associated with thrombus formation within or beyond the tumor capsule [9, 21].

Angio-invasive tumors have a high chance to metastasize hematogenously to bone and lungs. Follicular carcinomas lack multifocality and do not invade lymphatics. The tumor grows as a combination of solid, trabecular and follicular growth patterns. The metastatic lesions from follicular carcinoma are histologically similar to the primary neoplasm in the thyroid [4]. It can also be deceptively bland and mimic normal thyroid tissue.

Tumors with limited focal capsular and / or vascular invasion that are apparent only on histological examination are termed as 'Minimally invasive follicular carcinomas' [2, 22]. 'Follicular tumors of uncertain malignant potential' are designated, if the presence or absence of invasion is not certain or unequivocal, [2].

MEDULLARY CARCINOMA:

Medullary carcinoma is a malignant tumor of the thyroid gland, with evidence of parafollicular C cell differentiation. It comprises about 10% of all malignant thyroid malignancies [4]. Calcitonin secretion is characteristic. It

occurs in the setting of several inherited cancer syndromes including multiple endocrine neoplasia (MEN) syndromes. Medullary carcinoma can exhibit trabecular, insular or sheet like growth patterns traversed by delicate fibro vascular septa. The cells are small with stippled chromatin. Amyloid deposits are seen separating the tumor cell nests. Tumor necrosis and mitotic figures are infrequent. Lymphatic invasion, extra thyroidal involvement by direct extension and intra glandular metastasis can be present [9, 11].

POORLY DIFFERENTIATED CARCINOMA:

Poorly differentiated thyroid carcinoma or insular carcinoma represents a heterogeneous group of malignant neoplasms, with varied growth patterns and biological behaviour. It grows in the form of large solid nests, punctuated by variable number of small abortive follicles with a thin fibro vascular septa and retraction artifact around. The cells are small, uniform with hyper chromatic or vesicular nucleus and variable mitotic activity. Prominent vascularization, coagulative necrosis, infiltrative growth pattern and obvious vascular invasion are characteristic [2].

Apart from tumor stage, tumor necrosis, mitotic count of more than 3 per 10 high power fields and the age more than 45 years have been reported as being significantly associated with the aggressive biological behaviour of the disease [9, 17].

ANAPLASTIC CARCINOMA:

Anaplastic carcinoma or undifferentiated thyroid carcinoma accounts for about 5 - 10% of thyroid malignancies. The tumor is usually seen in iodine deficient areas and in elderly patients with a rapidly enlarging mass [2]. Anaplastic carcinoma exhibits a wide spectrum of morphologic patterns and cell types, predominant being epithelioid cells, spindle cells and giant cells. Focal squamoid differentiation and osteoclast like giant cells can be evident. The tumor also shows frequent mitoses, extensive coagulative necrosis and marked degree of invasion into the surrounding soft tissues [2]. Prominent vascularization and vascular invasion are common with obliteration of the vascular lumina. Distant metastasis is frequent and behaves in an aggressive fashion. A pre-existing well differentiated thyroid neoplasm, more often follicular or papillary carcinoma is usually seen in many, if not in most of the undifferentiated carcinomas [11, 17, 22].

TNM CLASSIFICATION AND STAGING OF THYROID CARCINOMAS [2]:

A staging and grading system is a standard way for the cancer care team to summarize how large a cancer is and how far it has spread. The TNM system is endorsed by the International Union against Cancer (UICC) and the American Joint Commission on Cancer (AJCC) and is commonly used.

T- PRIMARY TUMOR

TX Primary cannot be assessed.

T0 No evidence of primary tumor.

T1 Tumor 2 cm or less in greatest dimension, limited to thyroid.

T2 Tumor more than 2 cm but not more than 4 cm in greatest dimension, limited to thyroid.

T3 Tumor more than 4 cm in greatest dimension, limited to thyroid or any tumor with minimal extra thyroidal extension (e.g. extension to sternothyroid muscle or perithyroid soft tissues) limited to the thyroid.

T4a Tumor extends beyond the thyroid capsule and invades any of the following: subcutaneous soft tissues, larynx, trachea, esophagus, recurrent laryngeal nerve*

T4b Tumor invades prevertebral fascia, mediastinal vessels, or encases carotid artery*

T4a* (Anaplastic carcinoma only) Tumor (any size), limited to the thyroid**

T4b* (Anaplastic carcinoma only) Tumor (any size), extends beyond the thyroid capsule***.

Notes: Multifocal tumours of all histological types should be designated (m).

* All Anaplastic/undifferentiated thyroid carcinomas are considered T4.

** Intrathyroidal anaplastic carcinoma – considered surgically resectable.

*** Extra thyroidal anaplastic carcinoma – considered surgically unresectable.

N-REGIONAL LYMPH NODES

NX Regional lymph nodes cannot be assessed.

N0. No regional lymph node metastasis.

N1 Regional lymph node metastasis.

N1a Metastasis to Level VI (pretracheal, paratracheal and prelaryngeal / Delphian) lymph nodes.

N1b Metastasis to unilateral, bilateral or contra lateral cervical or superior mediastinal lymph nodes.

M – DISTANT METASTASIS

MX Distant metastasis cannot be assessed.

M0 No distant metastasis

M1 Distant metastasis

STAGE GROUPING:

Separate stage groupings are recommended for papillary and follicular, medullary and anaplastic/ undifferentiated carcinomas. Unlike most other

cancers, thyroid carcinomas are grouped into stages in a way that considers both the subtype of the neoplasm and the patient's age [2].

Papillary or Follicular under 45 years

Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1

Papillary or Follicular, 45 years and older and Medullary of any age

Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1a	M0
Stage IVA	T1, T2, T3	N1b	M0
	T4a	N0, N1	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Anaplastic/ Undifferentiated (all are considered stage IV)

Stage IVA	T4a	Any N	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

GRADING:

Tumor grading in thyroid malignancies is of little significance as more than 95% of cases are well differentiated using standard grading criteria [2]. However tumor necrosis, vascular invasion, increased mitoses and marked nuclear atypia have been associated with a less favorable prognosis in papillary carcinoma [17]. Certain variants such as tall cell variant and diffuse sclerosing variants are also associated with an aggressive clinical behaviour [9, 11]. Biological behaviour of follicular carcinoma can be predicted based on tumor size, local extension and presence of distant metastasis [18]. Medullary carcinoma with necrosis, squamous metaplasia and distant metastasis are associated with poor survival [23].

CD15 (Leu M1): A GENERAL REVIEW

Leu-M1, a blood group antigen was first reported as reactive with the membrane-related trisaccharide fucosyl-*N*-acetyl lactosamine on myelo-monocytic cells, where the epitope was known as *X-Hapten* or *Lewis-X*. The gene is located on chromosome 11 in the region q12 [3].

CD15 (Cluster differentiation antigen) is a complex cluster of cell surface glycoproteins and glycolipids having a common terminal pentasaccaride known as Lewis X (Le ^{x}) antigen. It is implicated in cell to cell adhesion mediated by carbohydrate-specific ligands. The core proteins and lipids differ in the different cell types, as there is marked variability in the binding of the

antibodies within and among them [24]. CD15 plays a role in increasing neutrophils adhesion, mediating phagocytosis, bactericidal activity by stimulation of degranulation, respiratory bursts and chemo taxis [25].

CD15 is also a haemopoietic differentiation antigen expressed on most terminally differentiated myeloid cells and Langerhans' cells [26]. It is also found in epithelial cells such as Breast (secretory epithelium), Kidney (proximal tubular cells), Lung and Intestinal tract (including Paneth cells). In the Brain, CD15 is constantly expressed in astrocytes and variably in Oligodendrocytes and Neurons. Complex glycoproteins containing fucose (such as Leu-M1) have been identified in epithelial cells lining the intestinal walls in early embryogenesis [27].

The CD15 antigen is present in more than 95% of the mature peripheral blood eosinophils and neutrophils and is also present in low density on circulating monocytes but not on basophils or lymphocytes [26]. In lymphoid tissue, CD15 reacts with Reed-Sternberg cells of Hodgkin's disease and with granulocytes [27]; however, CD15 reacts with a few tissue macrophages and does not react with dendritic cells. Antibodies to Leu-M1, like most CD 15 antibodies, developed are immunoglobulin M antibodies that recognize a specific sugar sequence in the complex glycolipids and glycoproteins such as lacto-N-fucopentaose III ceramide in the cells [25].

CD15 is expressed in varying proportions of epithelial derived tumors such as adenocarcinoma, renal cell carcinoma, and embryonal carcinoma and also occasionally in large cell lymphomas of both B and T cell phenotypes. These antigens are useful in the classification of leukemia, in distinguishing Hodgkin disease from non- Hodgkin's lymphoma, in further sub typing of Hodgkin lymphoma, and in differentiating metastatic adenocarcinoma from malignant mesothelioma. CD 15 a marker for murine pluripotent stem cells, in which it plays an important role in adhesion and migration of the cells in the preimplantation embryo [27].

Activation of CD15 results in cell activation of monocytes [28]. Sui et al suggested that E-selectin interaction with sialyl- CD15 may predominately mediate initial monocyte tethering, while concomitant or subsequent engagement of the nonsialylated form then mediates cell activation [28]. A role in cell-cell recognition during neuronal development is suggested by the observation that monoclonal antibodies against Lewis X inhibit the adhesion of cerebellar granule cells to astrocytes and block neurite outgrowth. CD15 containing molecules are also secreted in the neural tissues and have been shown to bind with Wnt-1, suggesting that CD15 may bind and present growth factors important for the proliferation and self-renewal of neural progenitors during embryonal life [25].

CD15 EXPRESSION IN THYROID CARCINOMAS:

For most thyroid tumors, a diagnosis of thyroid malignancy can be reached by morphological assessment alone. As of now in India, the diagnosis of papillary thyroid carcinoma is based on nuclear features. However, these features may be seen in some benign lesions too. Invasion of the capsule and/ or vascular channels represent diagnostic criteria for follicular carcinoma. Many a times, it is difficult to confirm by morphology alone [2]. At the same time, in tumors exhibiting unusual patterns or for confirmation of a diagnosis and to establish the prognosis, Immunohistochemical study becomes essential.

Blood group antigen (Lewis X) expression has been described in fetal tissues and tumorous tissues. They are present only in the initial embryologic developmental stages and are absent when organ maturation is completed. Synthesis of blood group antigen in the thyroid tissue is considered as 'oncofetal' as they are not detected in the normal adult organ. Carcinomas of the thyroid gland show a significant increase in expression of this antigen possibly secondary to oncogenic transformation and subsequent increased production of Lacto-N-fucopentaose III ceramide [24]. However, no correlation exists between antigen expression and degree of differentiation of the tumor. Anaplastic carcinomas were found to be negative for CD15 indicating the loss of these epitopes upon high grade malignant transformation [29].

The degree of epithelial Leu-M1 expression was significantly related to the biological behaviour of papillary and medullary thyroid neoplasms. Mortality was found to be 17 times more frequent among papillary tumors with marked positivity (>15%). Among medullary carcinomas, local recurrences occurred 2.9 times and death resulting from tumor occurred 4.3 times more frequently for intensely stained neoplasms as compared to slightly immunoreactive or unstained neoplasms [30].

It is established by Ito et al that, blood group related antigens like CD15 are expressed exclusively in papillary carcinoma of the thyroid gland [31]. Many studies have evaluated CD 15 expression in medullary thyroid carcinomas and follicular neoplasms as well and it was observed that its expression is more likely to be found in tumors with metastases than in those without metastases [32]. Van Hoeven et al illustrated that papillary thyroid carcinomas were the most immunoreactive for the blood group antigens and its expression in medullary carcinoma may be related to a biologically aggressive behaviour [33].

Larena et al reported a significant and progressive increase in expression of blood group antigens in carcinomas of the thyroid gland of higher grade and stage with no expression in normal thyroid tissue [34]. A significant higher level of recurrence and metastasizing capacity were observed by them in the neoplasms with significant blood group antigen expression [34].

A study reported by Mai et al states that CD 15 expression was specific although not sensitive to diagnose papillary thyroid carcinoma. This study revealed that CD 15 immunostaining in combination with CK 19 or HBME can achieve a sensitivity of 95% and specificity of 90% for the diagnosis of papillary carcinoma and hence will clarify the histological type in problem cases [35].

CD15 immuno reactivity may be helpful in the histological differential diagnosis between benign lesions and well differentiated thyroid neoplasms as well, and increased CD15 reactivity in malignant thyroid tumors reflects changes in thyroid follicular epithelial cell conjugates related to malignant transformation [36]. High grade expressions of these antigens were observed among the patients who presented with Masumane- Davidsohn phenomenon, where the tumor cells lack the expression of ABO blood group antigens. This was interpreted by Campora et al as a result of blockade in A and B antigen formation and the subsequent production of Leu antigens in the tumor cells [37].

As overlapping morphological patterns observed in various benign and carcinomatous lesions can limit the evaluation, Van Hoeven et al identified that CD15 can be used as a less sensitive but more specific marker to classify such morphologically unequivocal lesions [33].

Miettinen et al found a marked epithelial Leu M1 immunoreactivity in papillary thyroid carcinomas at an advanced stage of disease [38]. These findings

suggest that, CD15 positivity is associated with a poorer prognosis and provides significant prognostic information for patients with papillary carcinoma of the thyroid gland [38]. Schroder et al suggested that CD 15 positivity is associated with a poor prognosis. The degree of epithelial Leu- M1 expression was significantly related to the clinical course of papillary neoplasms. A good correlation was found between Leu-M1 immunoreactivity in papillary carcinomas with advanced pathological stage and cancer related deaths [39].

CD15 immunoreactivity may be helpful to assist differential diagnosis of thyroid carcinoma from benign thyroid diseases. Leu-M1 positive cases exhibited more advanced pathological stage, lymph node metastases and a greater incidence of tumor associated mortality than Leu-M1 negative cases [40]. Willgeroth et al found a poor post operative course of disease in patients with Leu-M1 positive tumor cells in comparison to Leu-M1 negative tumors [41].

Studies by Ostrowski and Merino emphasized that CD15 expression can be used as an indicator of advanced stage of the disease. The study also revealed diffuse Leu-M1 staining in all cases of tall cell variant of papillary carcinoma and typically exhibited a strong cytoplasmic to membranous staining pattern[42]. Strong expression of CD 15 in the tall cell variant correlates well with the aggressive clinical behaviour of these tumors compared to usual papillary carcinomas [9, 11, 43].

Neuhold et al found a significantly higher epithelial CD 15 positivity in the group with size greater than 4 cm and was also found exclusively in the presence of lymph node metastases [44]. No substantial difference in immunostaining was seen between primary thyroid neoplasms and metastatic or recurrent lesions. However, it was suggested that in patients particularly with tumor recurrences, CD 15 immunostaining may be of clinical relevance for the selection of patients in whom a more radical surgical approach would be justified.

MATERIALS AND METHODS

MATERIALS AND METHODS

All those cases diagnosed as a thyroid neoplasm between the period of Jan 2006 to Sep 2009 in the Department of Pathology, PSG Institute of Medical Sciences and Research, Coimbatore were included in the study.

The clinical details of these cases were taken from the medical records department of PSG IMSR after obtaining permission from the authorities. Age, sex, clinical presentation, hormone status and follow up were obtained by analyzing the case records.

The slides of all the cases were screened and were analyzed for the following: the type of neoplasm, nuclear features, invasion into the capsule and vascular spaces, extra thyroidal extension, lymph node metastases, mitoses, necrosis and presence or absence of Amyloid. Paraffin blocks of those sections which had high tumor density were chosen for the study. Paraffin blocks of slides which showed tumor with large areas of hemorrhage, cystic change and necrosis were not considered for the study. The T and N status of the malignant neoplasms was also noted for staging.

4μ thick sections from the chosen paraffin blocks were made. They were mounted onto the glass slides and were stained with routine hematoxylin and eosin stain. These slides were reassessed for adequacy. Fresh sections from

the blocks were cut again at 5µ thickness and taken on to a Poly-L-Lysine coated slide.

Tissue blocks of normal kidney were taken from the nephrectomy specimens received. The blocks were then cut to 5µ thickness and taken on to a Poly-L-Lysine coated slide to serve as a positive control for CD15 [3].

Immunohistochemistry for the detection of CD15 expression was done using the super sensitive polymer – HRP detection system along with appropriate control (normal kidney). 3, 3'diaminobenzidine tetra hydrochloride (DAB) was used as the chromogen. The procedure followed is described below.

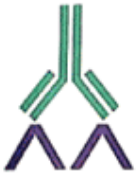
METHOD: The supersensitive polymer – HRP detection system

ANTIBODY: CD15 (Biogenex, clone BRA4F1)

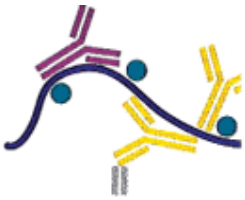
PRINCIPLE: Antigens in tissues and cells was detected by a two stage process: the binding of the primary antibody to specific epitopes and subsequent detection of this bound antibody by a colorimetric reaction using a substrate chromogen [45].

The method is based on the utility of compact dextran polymer to which multiple molecules of the enzyme (Horse radish peroxidase) are attached to each linker secondary antibody (specific for the unconjugated primary antibody). A primary antibody (CD15) for the antigen to be localised is first employed. This is followed by the addition of dextran polymer with multiple

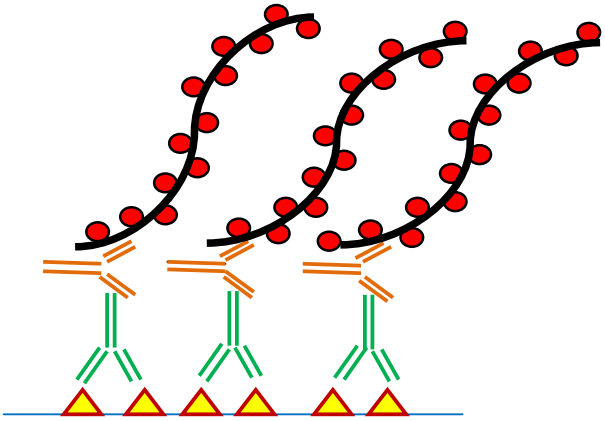
conjugated secondary antibodies. Multiple secondary antibodies react with different antigenic sites on the primary antibody, thereby increasing the signal amplification with the use of a suitable chromogen 3, 3'diaminobenzidine tetra hydrochloride (DAB) [24, 45].



Primary antibody



Secondary antibody



--- Polymerase enzyme

--- Secondary antibody

--- Primary antibody

---- Tissue antigen

---- Dextran polymer

ANTIGEN RETRIEVAL: Various methods are used to expose the antigenic sites (epitopes) that may be unexposed (masked) during routine processing due to formation of cross linking by the action of formalin [45]. The methods to unmask the epitopes include digestion with a variety of proteolytic enzymes, microwave heating, and lastly exposure to the combined effects of heat and pressure in a stainless steel pressure cooker as is said to be more uniform. It can recover almost full antigenicity. In this study, antigen retrieval was carried out using pressure cooker for 10 min, using Citrate Buffer at a pH of 6.0.

REAGENTS:

1. Citrate buffer at pH of 6.0. It was prepared by dissolving Tri sodium citrate (2.94g) in 1000ml of distilled water and 5ml of 1 N Hcl.

2. 3% H2O2 in distilled water to block endogenous peroxidase activity.

3. Phosphate buffered saline (PBS) with a molarity of 0.01M and the pH value of 7.6. It was prepared by dissolving the following substances in 1000 ml of distilled water.

Na2HPO4 Dibasic sodium phosphate, anhydrate	17.5g
KH2PO4 Monobasic potassium phosphate, anhydrous	2.5g
NaCl Sodium chloride	17.0g

4. Blocking reagent- contained casein in PBS with 15mM sodium azide. This was used to blocks non specific protein binding.

5. Primary antibody against CD15 is a mouse monoclonal antibody supplied in liquid form. (Biogenex, clone BRA4F1).

6. Poly HRP reagent- anti-mouse and anti-rabbit IgG complex linked to Horse radish peroxidase enzyme.

7. DAB (3, 3'Diamino Benzidine tetra hydrochloride) - Chromogen.

It offers great sensitivity as an HRP calorimetric chromogen and provides insoluble permanent coarse brown precipitate.

8. Harris hematoxylin as counter stain.

9. DPX (Distrene dibutyl phthalate Xylene) - Mountant.

PROCEDURE:

1. Sections were cut at 4μ thickness and taken on a egg albumin coated slide.

2. Routine Hematoxylin and eosin stain was done and reassessed.

3. Sections were cut at 5 micron thickness and taken on a Poly-L-Lysine coated slide.

4. Immunohistochemical Stain with CD15 antigen was done as follows

a. Slides were dewaxed and dehydrated in graded alcohol.

b. Heat induced antigen retrieval in citrate buffer at pH 6.0 using pressure cooker for 10 minutes

c. Washed in PBS buffer at pH 7.6 for 5 minutes twice

d. Slides were immersed in 0.3% H2O2 solution for 20 minutes to block endogenous peroxidase activity.

e. Washed in PBS buffer thrice each 5 minutes

f. Slides incubated with blocking solution for 10 minutes to block non-specific protein binding.

g. Washed in PBS buffer thrice.

h. Slides were incubated with CD 15 primary antibody for 1 Hr.

i. Enhancer was applied to the sections for 30 minutes to enhance the signal intensity.

j. Washed in PBS buffer thrice.

k. Slides were incubated with polymer Horse radish peroxidase reagent.

l. Washed in PBS buffer thrice.

m. Diamino Benzidine (DAB) is applied.

n. Washed in PBS buffer thrice.

o. Sections counter stained with Harris hematoxylin for 1 minute.

p. Washed in tap water.

q. Sections cleared in Xylene and mounted with DPX mountant.

Tumors cells were scored positive if there was Brown Cytoplasmic peri nuclear dot like staining or membranous accentuation in more than 10% of the neoplastic cells. Scoring is done in the well stained area with no interference by nonspecific staining background.

Immunoreactivity is evaluated as follows:

(-) absolutely no cells show immunostaining.

(+/-) <10% of neoplastic cells show weak or focal reactivity.

(+) 10 – 50% of neoplastic cells show moderate to strong reactivity.

(++) > 50% of neoplastic cells with moderate to strong reactivity.

RESULTS

RESULTS

The Department of Pathology, PSG Institute of Medical Sciences and Research has received 15739 biopsy specimens over a period of 3 years and 9 months from 1st Jan 2006 to 30th Sep 2009, of which 266 were Thyroidectomy specimens. The total numbers of malignancies reported in our institute during this study period were 2953, out of which thyroid neoplasms were 64 with an overall incidence of 2.16 % of the total cancer cases ranging from 1.65% to 2.72% during the study period.

TABLE II: Incidence of thyroid neoplasms during the study period.

Year	Total no of neoplasms diagnosed	No of Thyroid neoplasms	Incidence of thyroid neoplasms
2006	625	17	2.72%
2007	819	19	2.32%
2008	847	14	1.65%
Sep 2009	662	14	2.11%
Total	2953	64	2.16%

Based on the inclusion criteria, the number of cases in the study group was 50. Out of these 50 cases, 30 were papillary thyroid carcinoma, 17 were follicular neoplasms [follicular adenoma (12), minimally invasive follicular

carcinoma (2) and 1 each of follicular neoplasm of uncertain malignant potential, microfollicular adenoma of signet ring cell type and Hurthle cell adenoma), two cases of medullary carcinoma and a single case of anaplastic carcinoma with coexisting follicular carcinoma. Minimally invasive follicular carcinoma, follicular neoplasm of uncertain malignant potential, microfollicular adenoma of signet ring cell type, Hurthle cell adenoma and anaplastic carcinoma were placed under miscellaneous category. The breakup and incidence of various types of thyroid neoplasms during the study period is as shown in the table III.

TABLE III: Distribution of thyroid neoplasms during the study period.

Year	Total no of Thyroid neoplasms	Papillary carcinoma	Follicular adenoma	Medullary carcinoma	Miscellaneous
2006	17	9 (53%)	4 (23.5%)	1 (5.9%)	3 (17.6%)
2007	19	12 (63.2%)	5 (26.3%)	1 (5.3%)	1 (5.2%)
2008	14	11 (78.6%)	2 (14.3%)	0	1 (7.1%)
Sep 2009	14	9 (64.3%)	4 (28.5%)	0	1 (7.1%)
Total	64	41 (64%)	15 (23.4%)	2 (3.1%)	6 (9.4%)

THYROID NEOPLASMS- AGE WISE DISTRIBUTION:

The age group ranged from 20 to 70 years with a mean age of 45 years. Papillary thyroid carcinoma was predominantly seen in the second and fourth decades. Follicular neoplasms dominated in the third decade. Medullary carcinoma was seen in the third and sixth decades of life. A single case of anaplastic carcinoma in our study was in a 40 year old female, contrary to what is described in the literature (i.e., usually seen in the elderly). Table IV shows the age wise distribution of patients among various neoplasms of thyroid.

TABLE IV: Distribution of various thyroid neoplasms age - wise

Age in yrs	Papillary carcinoma	Follicular neoplasm	Medullary carcinoma	Anaplastic carcinoma
20 - 30	11	2	0	0
31 – 40	3	7	1	1
41 – 50	9	5	0	0
51 – 60	5	3	0	0
61 – 70	2	0	1	0
71 - 80	0	0	0	0
TOTAL	30	17	2	1

THYROID NEOPLASMS- SEX WISE DISTRIBUTION:

Of the 50 cases, 13 were males and 37 were females giving rise to an M: F ratio of 1:2.8. Sex distribution among various neoplasms was also observed. Out of 30 cases of papillary carcinomas, 6 were in males against 24 females with an M: F ratio of 1:4. Both the cases of medullary carcinoma occurred in males and that of the anaplastic carcinoma were in a female. Among follicular neoplasms, 12 cases were females and 5 were males which also showed a female predominance. Table V and chart I shows the sex distribution of thyroid neoplasms of various types, which clearly depicts female predilection of papillary and follicular thyroid carcinomas in our study group as comparable with the literature.

CHART I: Distribution of thyroid neoplasms sex – wise:

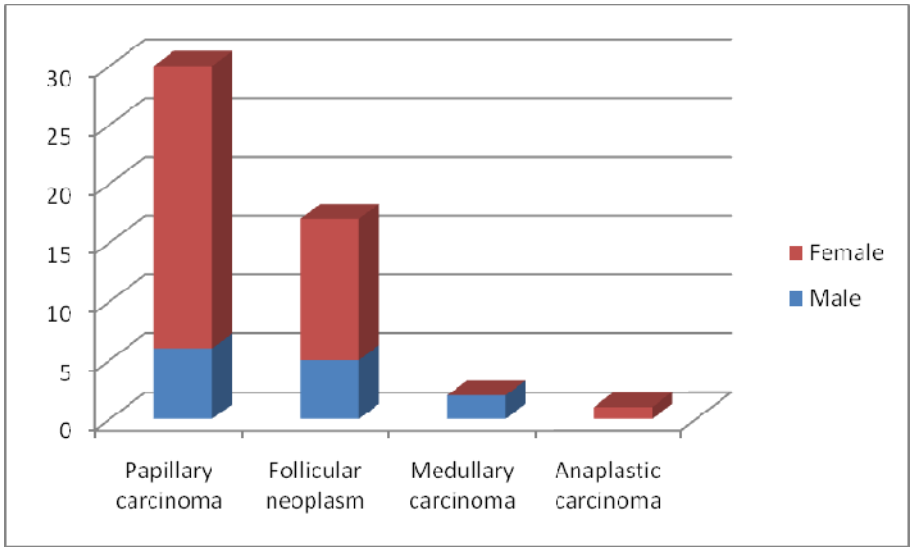


TABLE V: Distribution of various thyroid neoplasms sex – wise:

Sex	Papillary carcinoma	Follicular neoplasm	Medullary carcinoma	Anaplastic carcinoma
Male	6	5	2	0
Female	24	12	0	1

THYROID NEOPLASMS- HORMONE STATUS ANALYSIS:

Hormone status was obtained for these cases from the case records. Free T3 and T4 levels and TSH levels were taken and interpreted. Out of 50 patients, 42 patients were in euthyroid state accounting for about 84% and three each in hypothyroid and hyperthyroid status. For two patients, hormone statuses were not available as shown in Table VI. Of the two cases of medullary carcinoma encountered in the study, serum calcitonin level was found to be elevated (2ng/ml) pre-operatively.

TABLE VI: Hormone status analysis of thyroid neoplasms:

Hormone status	Euthyroid	Hypothyroid	Hyperthyroid	Not available
No of cases	42	3	3	2

THYROID NEOPLASMS- GROSS PATTERN ANALYSIS:

Sizes of various thyroid neoplasms studied ranged from 0.4 cms to 11.5 cms. Table VII shows the size wise distribution of cases among various types of thyroid neoplasms.

TABLE VII: Distribution of various thyroid neoplasms size – wise:

Size	Papillary carcinoma	Follicular neoplasm	Medullary carcinoma	Anaplastic carcinoma
<2cm	13	1	0	0
2 – 4 cm	13	10	2	0
>4 cm	3	6	0	1
Not assessed	1	0	0	0

Out of 30 cases of papillary carcinoma, four were papillary microcarcinomas with sizes ranging from 0.4 to 1.0 cms. Cystic lesion with papillary structures along the cyst wall was found in six cases. Stromal bone formation was noted in one. Encapsulation was found in one of the cases. The tumor was multifocal in ten patients (33.3%) and two patients had lymph node involvement. Extrathyroidal extension beyond the thyroid capsule was found in three of the patients with papillary carcinoma, of which one patient had lymph node involvement in addition.

Amongst the follicular neoplasms of thyroid, all the lesions showed a thick fibrous capsule enclosing the nodule, with a thin rim of normal thyroid parenchyma around. One of the cases had a thin capsule. Cervical lymph node involvement was found in both the cases of medullary carcinoma and with necrosis in one. The lone case of anaplastic carcinoma in our study was 11.5 cms in size involving the right lobe and isthumus with extrathyroidal extension into the superior mediastinum. The tumor was found to be displacing the trachea with compression and also encasing the right common carotid artery by CT neck. The cut surface revealed areas of necrosis and hemorrhage. Bilateral cervical and mediastinal lymph nodes were also involved by the tumor.

THYROID NEOPLASMS- ANALYSIS OF MICROSCOPIC FEATURES:

Hematoxyllin and eosin stained sections were analysed for the following: architectural pattern, nuclear atypia, vascular invasion, capsular invasion, stromal sclerosis, mitoses, necrosis, associated chronic thyroiditis and coexisting other neoplasms. Apart from these, presence of amyloid deposits and squamous metaplasia were also studied in medullary carcinoma.

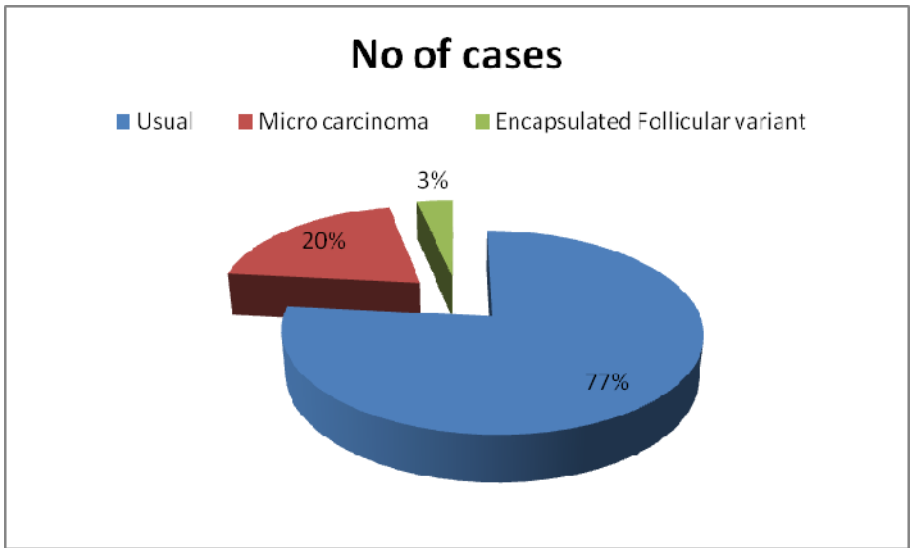
29 0f the the 30 cases of papillary carcinoma showed obvious papillary architecture (Fig. 1, 2), one of which showed in addition oncocytic cells (Fig. 3). One case which did not show papillary architecture showed a follicular architecture and was lined by cells with nucler features typical of a papilary carcinoma [encapsulated follicular variant (Fig. 5)]. Solid areas were noted in

foci in two of the cases. Four cases in our study were of micropapilary carcinoma (Fig. 4) and were discovered incidentally.. Mild nuclear atypia and focal capsular invasion was noted (Fig. 6) in four of the cases. Table VIII and chart II depict the various patterns of papillary carcinoma reported in our study.

TABLE VIII: Histological distribution of papillary carcinoma thyroid:

Papillary Carcinoma	No of cases	Incidence (%)
Usual type	23	77%
Micro carcinoma variant	6	20%
Encapsulated Follicular variant	1	3%

CHART II: Incidence of classical type and variants of papillary carcinoma thyroid:



Two cases had cervical lymph node involvement at the time of diagnosis (Fig. 7). Stromal sclerosis was noted in sixteen of the cases with stromal bone formation in one (Fig. 8). Other thyroid lesions were found to coexist in 10 of the 30 cases. They are chronic thyroiditis seen in 30% cases (Fig 9), follicular adenoma (7%) and follicular neoplasm of uncertain malignant potential (3%).

TABLE IX: Histological distribution of follicular neoplasm:

Type of neoplasm		No of cases
Follicular adenoma	Usual type	12
	Micro follicular adenoma- signet ring cell type	1
	Hurthle cell adenoma	1
	Follicular neoplasm of Uncertain malignant potential	1
Follicular carcinoma	Minimally invasive follicular carcinoma	2

Among the 17 cases of follicular neoplasms, 15 cases were benign (folicular adenoma) and 2 were malignant. Of the 15 benign neoplasms, 12 were of usual type with a thick fibrous capsule (Fig. 12, 13) and 3 were variants which are microfollicular adenoma of signet ring cell type (Fig. 14, 15), hurthle cell adenoma (Fig. 16, 17) and a follicular neoplasm of uncertain malignant

potential (Fig. 18). Both the cases of follicular carcinoma showed minimal focal capsular invasion (Fig. 19). Table IX gives the details of follicular neoplasm and its variants.

In both the cases of medullary carcinoma, the cells were arranged predominantly in nests and sheets (Fig. 21, 22) with mitotic figures of about 2/ 25 high power fields in both of the cases. Both the cases of medullary carcinoma showed the presence of acellular amorphous eosinophilic deposits of amyloid in the stroma (Fig. 23). Presence of amyloid in the sections were confirmed using congo red stain (Fig. 24). Under polarised microscopy, apple green birefringence was observed (Fig. 25). Invasion of the thyroid capsule with capsular blood vessel invasion (Fig. 26) was noted in one and the other showed tumor at the surgical resection margins. Bilateral upper and deep cervical lymph node involvement was found in both the cases at the time of diagnosis (Fig. 27).

Anaplastic carcinoma exhibits prodominantly solid pattern of growth with marked nuclear pleomorphism, necrosis and increased mitoses of about 14 / 25 high power fields (Fig. 29, 30). A transition from follicular carcinoma was observed in foci (Fig. 31, 32). Invasion into the adjacent skeletal muscle bundles were also seen.

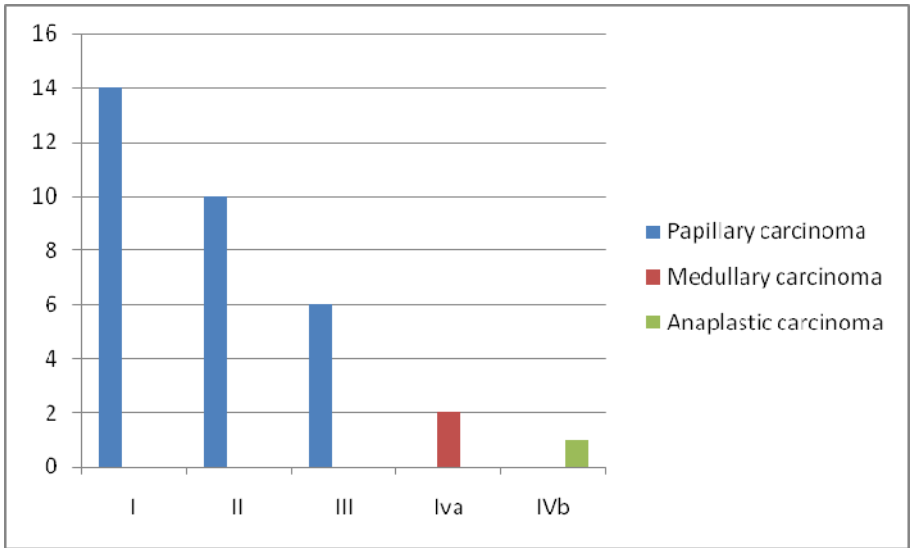
THYROID NEOPLASMS- ANALYSIS OF STAGE OF THE TUMOR:

Staging was done according to the TNM classification and staging of thyroid carcinomas, considering the age, size, lymph node involvement, extra thyroidal extension, type of neoplasm and presence or absence of metastases. Table X and chart III depict the distribution of thyroid neoplasms based on their stage at diagnosis. 80% 0f papillary carcinoma presented in stage I and II, which has a good prognosis. The remainder was in stage III. Stage IV was not observed in our study. Both cases of medullary carcinoma were in stage IV A and the anaplastic carcinoma was in stage IV B. as of now, there is no stage grouping described for minimally invasive follicular carcinoma. Hence it is not represented in the table.

TABLE X: Distribution of various thyroid neoplasms based on stage at diagnosis:

Stage	Papillary carcinoma	Medullary carcinoma	Anaplastic carcinoma
I	14	0	0
II	10	0	0
III	6	0	0
Iva	0	2	0
IVb	0	0	1

CHART III: Distribution of various thyroid neoplasms based on stage at diagnosis:



THYROID NEOPLASMS- IMMUNOHISTOCHEMICAL ANALYSIS OF EXPRESSION OF CD15 ANTIGEN:

Sections of thyroid neoplasms stained with CD 15 antibody were studied for the intensity of staining and percentage positivity of the tumor cells (scoring). The intensity of stainging were graded as mild, moderate and strong. Scoring was done by counting 500 cells in the well stained areas of high tumor load and a percentage of positivity was calculated accordingly. Areas with nonspecific staining if any, and hemorrhage are excluded, as blood cells like neutrophils and macrophages would also show CD 15 immuno marker expression.

Twelve of the 50 thyroid neoplasms of the study showed positivity for CD 15. Thus the incidence of CD15 expression in thyroid neoplasm in our study was 24%. Papillary carcinomas accounted for 66% of the overall positivity. Table XI details the incidence of CD15 expression in the various histological types of thyroid neoplasms.

TABLE XI: Incidence of CD15 expression in various types of thyroid neoplasms.

Type of neoplasm	Total No of cases	No of cases positive for CD15	Percentage positivity
Papillary carcinoma	30	8	26.7%
Follicular adenoma	15	2	13.3%
Medullary carcinoma	2	2	100%
Miscellaneous	3	0	0%
Total	50	12	24%

Of the 30 papillary carcinomas, 8 were CD15 positive (26.7%). Seven of the 8 cases showed focal membranous staining (Fig 10, 11), one of which also showed focal cytoplasmic dot like reactivity. One case showed diffuse membranous staining (moderate to strong). The staining score ranged from 26% to 100%.

Both the medullary carcinomas encountered in this study showed a Strong cytoplasmic dot like staining (Fig. 28) with 63% and 96% positivity. The lone anaplastic carcinoma seen in our study was negative for CD15 marker expression. CD15 expression was also seen in 2 of the 15 cases of benign folicular neoplasms, both of which showed focal cytoplasmic dot like staining (Fig. 20) and was about 53% and 67%.

TABLE XII: Incidence of CD15 expression in papillary carcinoma across various stages:

Stage	Total no of cases	No of cases positive for CD15	Incidence (%)
I	14	2	14.3%
II	10	3	30%
III	6	3	50%
IV	-	-	-
Total	30	8	27%

In cases of papillary carcinoma, CD15 immuno marker expression was more in patients with stage III and stage II disease and in the age groups of 45 and above (Table XII, XIII). CD 15 expression was very high in males. The scoring showed a general tendency for increased expression with increasing stage of the tumor. It was also found that, patients with capsular blood vessel

invasion, extra thyroidal extension, lymph node metastases had a high degree of marker expression in the tumor cells (> 50%). No generalizations could be drawn with regard to hormone status and CD15 expressivity as almost all of the cases were euthyroid.

TABLE XIII: Characteristics of papillary carcinoma thyroid which are positive for CD15:

S. No	Age / Sex	Stage	CD15 expression	Hormone status	Additional features
1	70 / M	II	100%	Euthyroid	Capsular blood vessel invasion
2	50 / M	III	72%	Euthyroid	Extra thyroidal extension
3	45 / F	III	61%	NA	Extra thyroidal extension, lymph node metastasis (4/13)
4	45 / F	II	58%	Euthyroid	_
5	46 / F	III	41%	Euthyroid	Multifocal
6	32 / F	I	28%	Euthyroid	_
7	41 /F	I	27%	Euthyroid	Multifocal, Stromal bone formation
8	27 / F	II	26%	Euthyroid	_

TABLE XIV: Characteristics of medullary carcinoma thyroid which are positive for CD15:

Age / Sex	Stage	CD15 expression	Hormone status	Additional features
67 /M	IV A	96%	Hypothyroid	Necrosis + Calcitonin high
38 / M	IV A	63%	Euthyroid	Necrosis – Calcitonin high

Table XIV shows that both the cases of medullary carcinoma of thyroid which were positive for CD15 showed >50% expression and had a similar advanced stage at presentation (Stage IV A).

Two of the 15 cases of follicular adenomas which showed positivity for CD 15 also showed >50% expression (Table XIV). Peculiarly both these cases showed a common unique histological finding i.e., focal clearing of nuclei and occasional nuclear grooves.

TABLE XV: Characteristics of follicular adenoma thyroid which are positive for CD15:

Age / Sex	CD15 expression	Hormone status	Additional features
48 / M	67%	NA	Focal clearing of nuclei and nuclear grooves
35 / F	53%	Hyperthyroid	

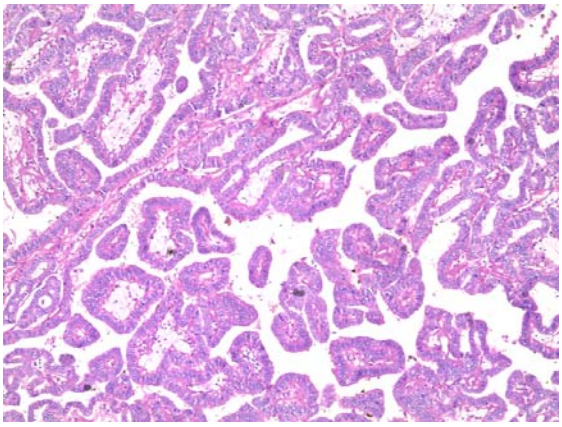


Fig 1: Papillary carcinoma. Complex arborising papillae with thin fibrovasculer core. H & E (10x).

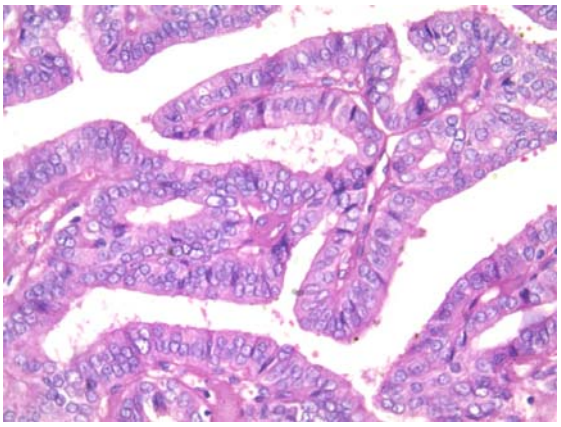


Fig 2: Typical nuclear features of Papillary carcinoma. H & E (40x)

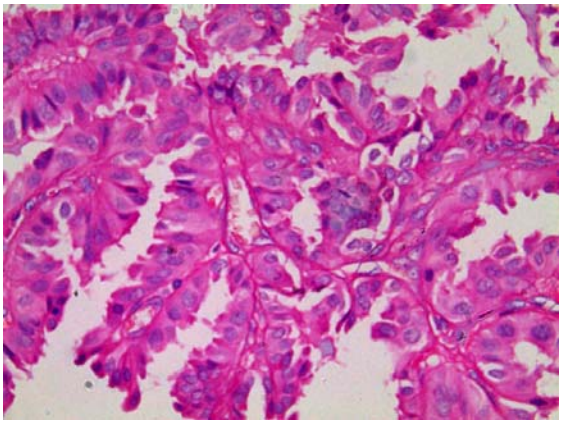


Fig 3: Papillae covered by oncocytic cells with with eosinophilic cytoplasm. H & E (40x)

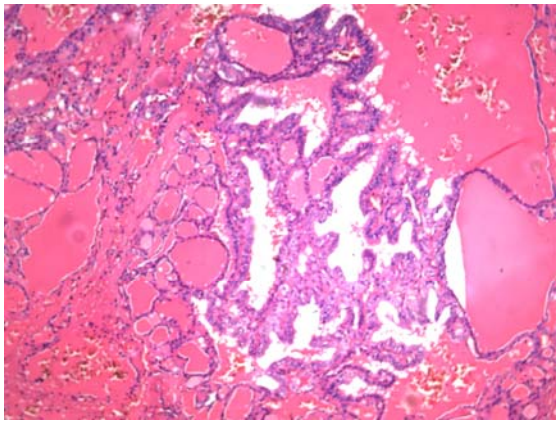


Fig 4: A focus of micropapillary carcinoma surrounding normal thyroid tissue. H & E (10x)

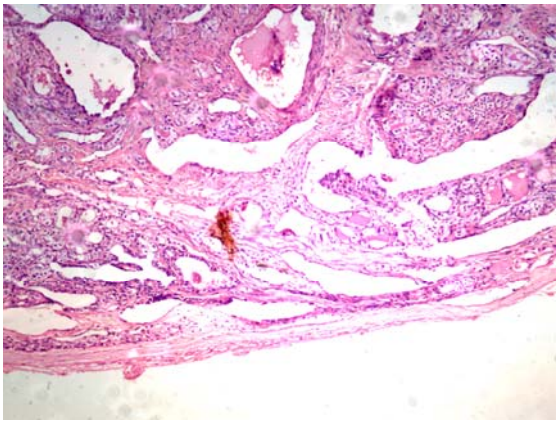


Fig 5: Encapsulated follicular variant of Papillary carcinoma showing a thin capsule and neoplastic cells in follicular pattern. H & E (10x)

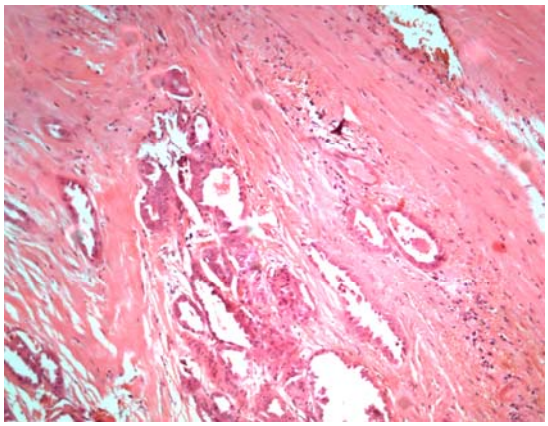


Fig 6: Papillary carcinoma with focal invasion into the capsule. H & E (10x).

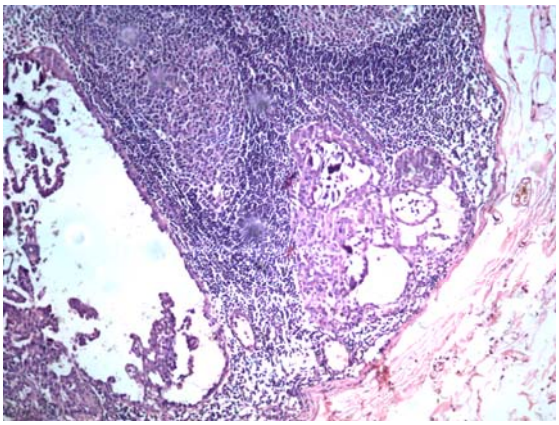


Fig 7: Lymph node showing metastatic foci of Papillary carcinoma. H & E (40X)

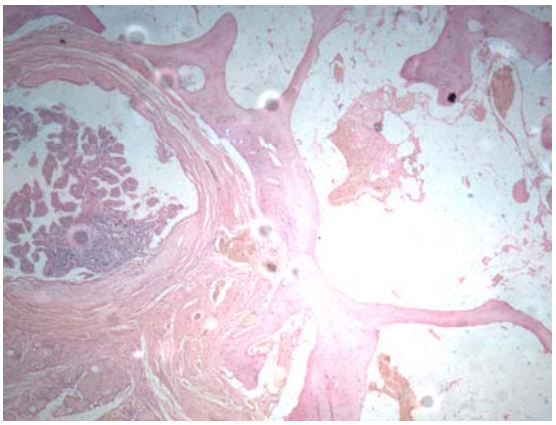


Fig 8: Extensive stromal sclerosis with bone formation in Papillary carcinoma. H & E (40X)

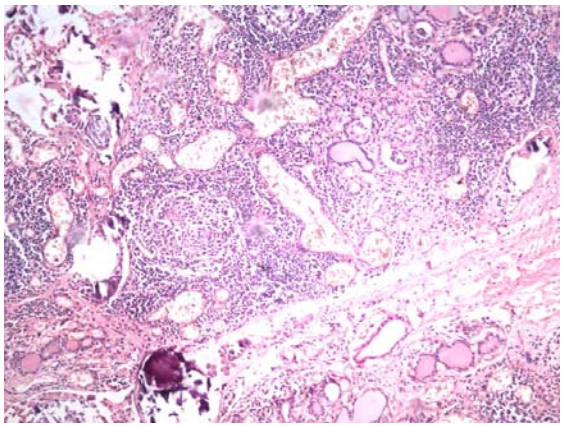


Fig 9: Lymphoid aggregates infiltrate the follicles. A few lamellated psammoma bodies are present. H & E (10x).

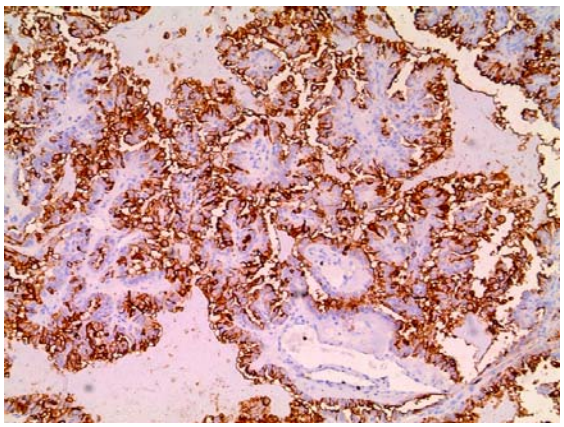


Fig 10: Papillary carcinoma showing diffuse strong membranous positivity for CD 15. IHC with hematoxylin counter stain (10x)

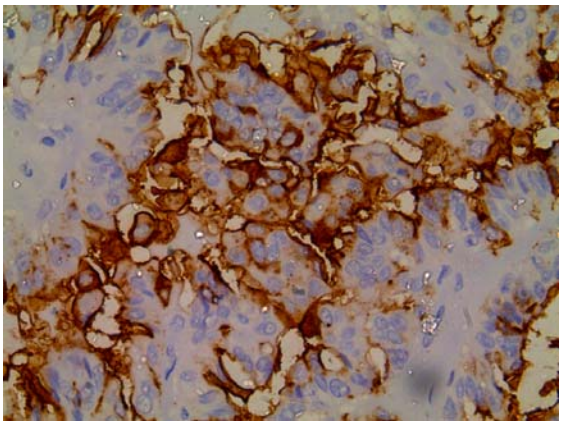


Fig 11: Diffuse strong membranous positivity of CD 15 in Papillary carcinoma. IHC with hematoxylin counter stain (40x)

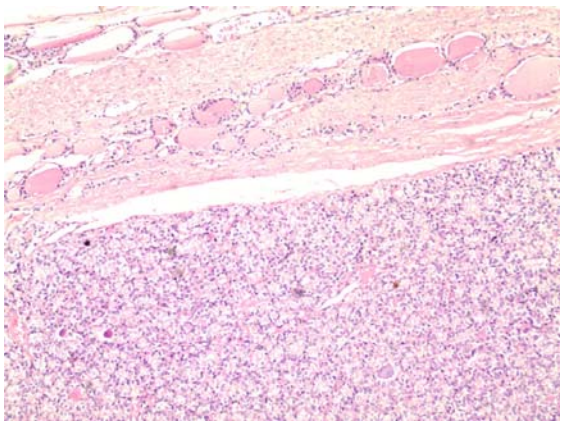


Fig 12: Follicular adenoma with a thick capsule and neoplastic cells in follicular pattern. H & E (10x)

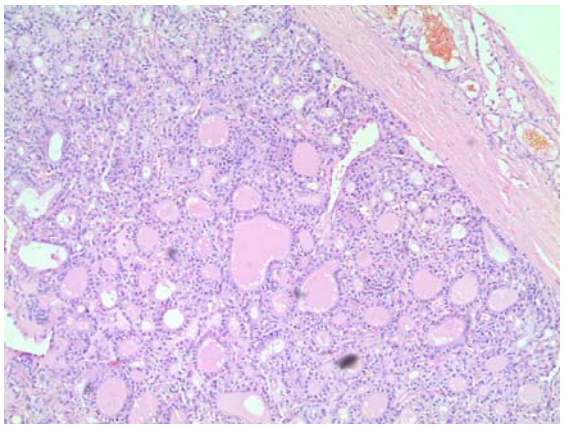


Fig 13: Encapsulation and neoplastic follicles of variable sizes in Follicular adenoma H & E (40x).

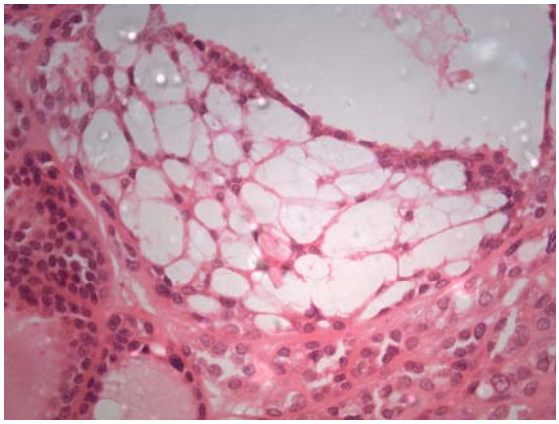


Fig 14: Collection of large vacuolated cells with eccentric nuclei and microfollicles in micro follicular adenoma- signet ring cell type. H &E

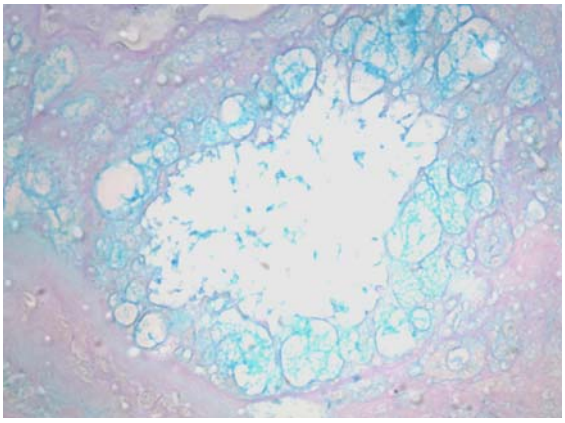


Fig 15: Signet ring like cells in Micro follicular variant of Follicular adenoma. AB-PAS (40x)

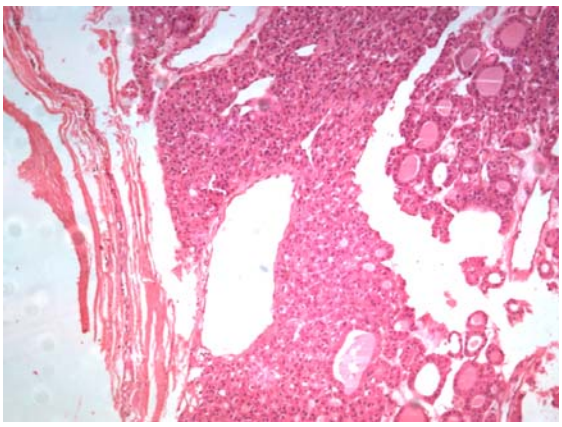


Fig 16: Hurthle cell neoplasm with a fibrous capsule and neoplastic follicles. H & E (10x)

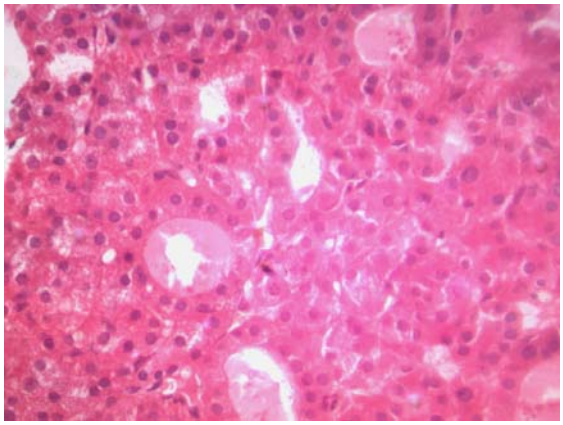


Fig 17: The follicles in Hurthle cell neoplasm lined by cuboidal cells with eosinophilic granular cytoplasm. H & E (40x)

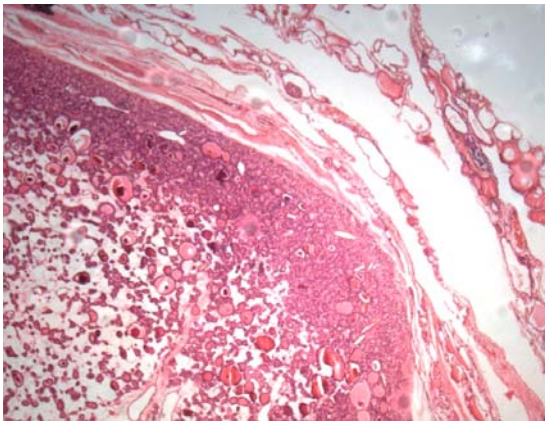


Fig 18: uncertain capsular invasion in a follicular neoplasm- Follicular neoplasm of uncertain malignant potential. H & E (10x)

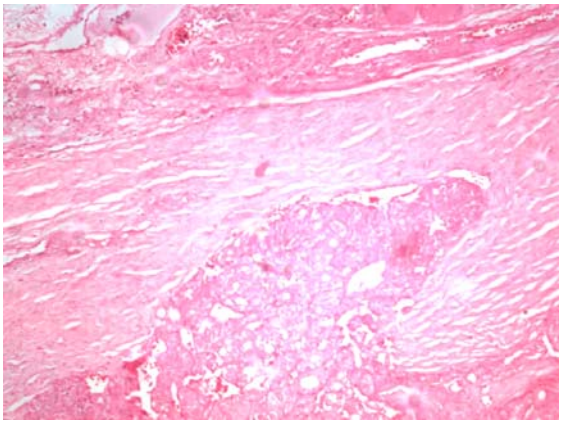


Fig 19: Follicular neoplasm with a small foci of limited capsular invasion. H & E (40x)

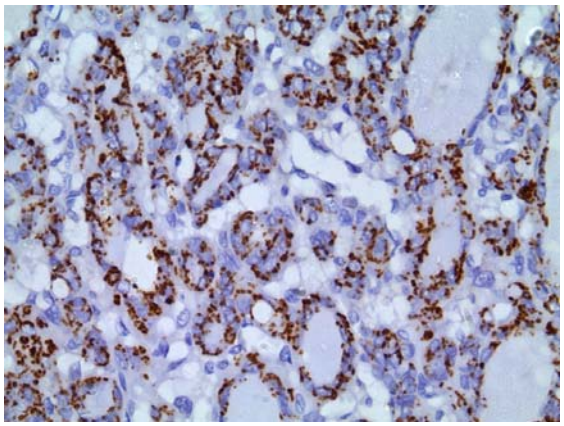


Fig 20: Follicular neoplasm showing strong dot like cytoplasmic positivity for CD 15 in more than 50% of the tumor cells, IHC with hematoxylin counter stain (40x)

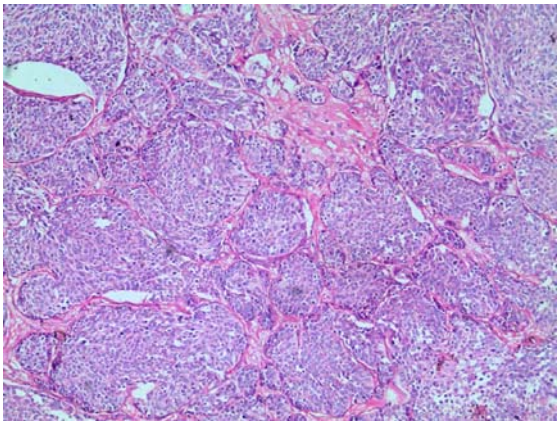


Fig 21: Medullary carcinoma showing nests and islands of tumor cells separated by fibrous stroma. H & E (10x)

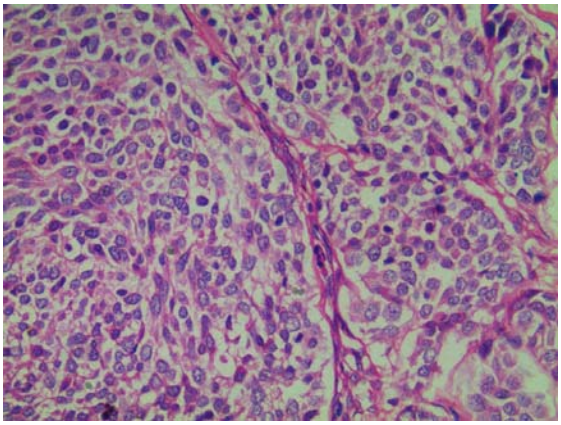


Fig 22: Nests of tumor cells with pale cytoplasm and round nuclei with stippled chromatin. H & E (40x)

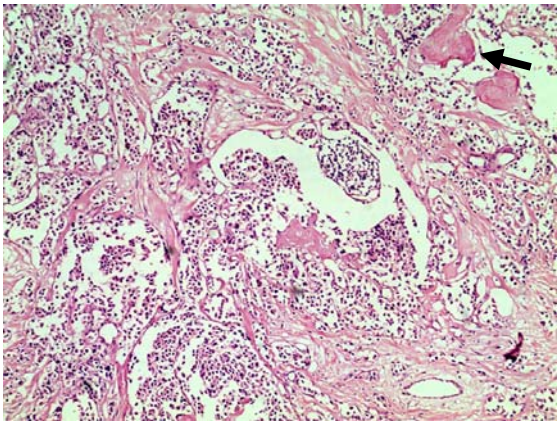


Fig 23: Medullary carcinoma showing nests of tumor ells. Homogenous pink acellular deposits are seen in the stroma (arrow). H & E (10x)

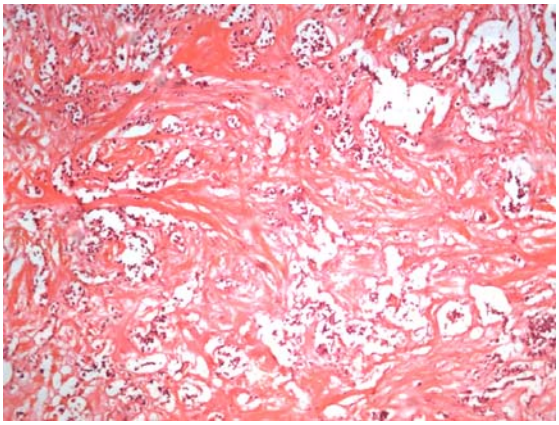


Fig 24: The acellular deposits separating the cell nests are congophillic. Congo red (40x)

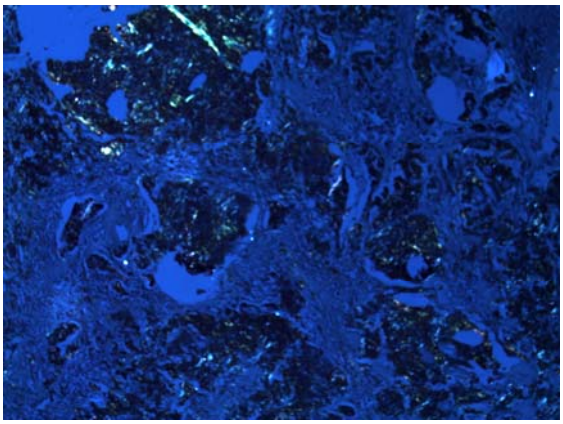


Fig 25: Medullary carcinoma Apple green birefringence of Amyloid deposits in polarizing microscopy. Congo red (10x).

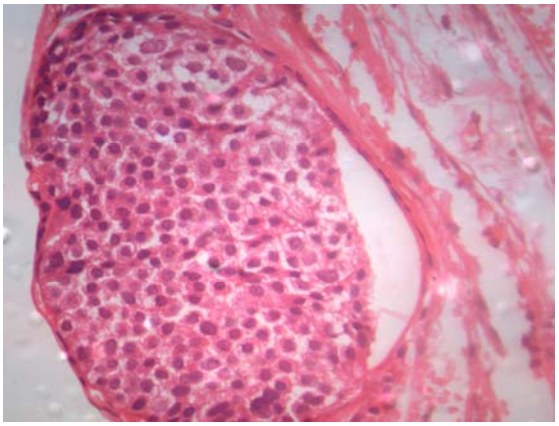


Fig 26: A blood vessel shows tumor cells of medullary carcinoma adherent to the endothelial lining. H & E (40x)

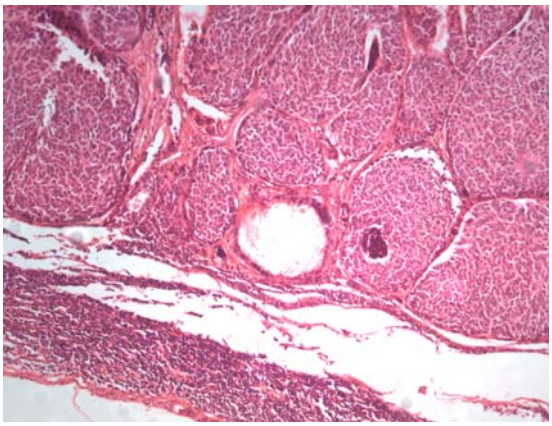


Fig 27: Medullary carcinoma replacing the lymph node. Lymphoid tissue with the subcapsular sinus is at the lower border of the field. H & E (10x)

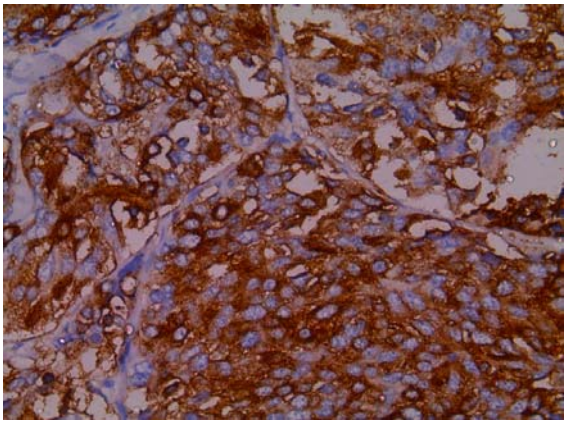


Fig 28: Medullary carcinoma shows strong granular cytoplasmic staining in most of the tumor cells. IHC with hematoxylin counter stain -CD 15 (40x)

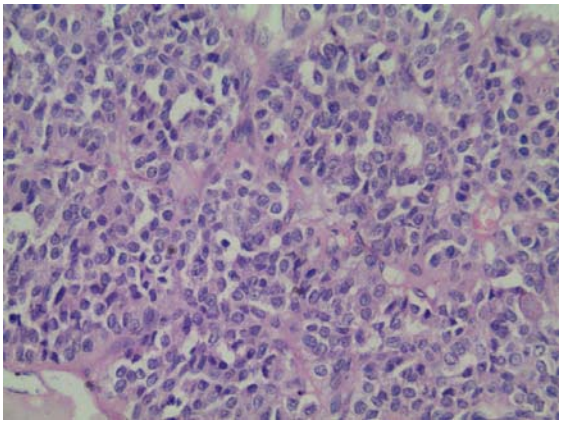


Fig 29: Follicular carcinoma with cells in solid sheets and follicles. H & E (40x)

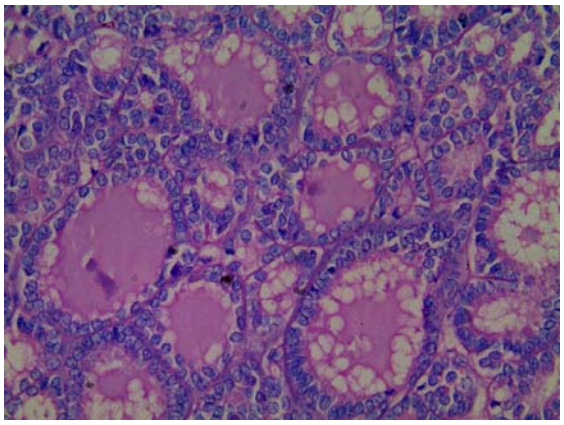


Fig 30. High magnifiction shows mild nuclear atypia and larger dark staining nuclei. H & E (40x)

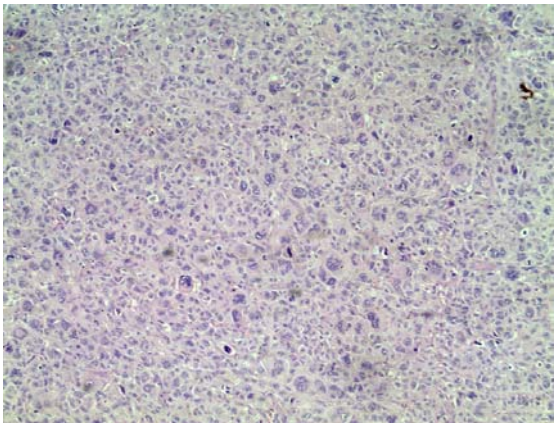


Fig 31: Anaplastic carcinoma shows large pleomorphic cells in solid sheets. H & E (10x)

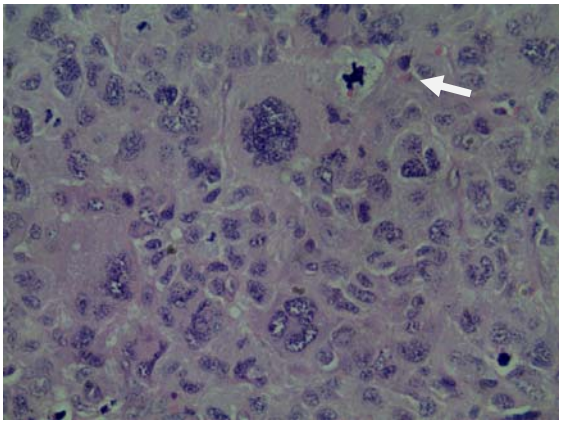


Fig 32. High magnifiction shows few bizarre cells multilobate nuclei. Increased mitotes with atypical forms are seen (arrow). H & E (40x)

DISCUSSION

DISCUSSION

Thyroid neoplasms are on the rise and pose a significant challenge to both pathologists and therapists. With increasing discoveries of variants of each type of malignancy (for eg: Follicular variant of papillary carcinoma, follicular carcinoma with minimal capsular invasion etc), it poses diagnostic challenge to the routine pathologists. Therapy too is confounded by the fact that the variants of these malignancies differ significantly in their clinical behaviour. There is a lot of research in progress to identify prognostic indicators which could solve the clinicians' dilemma. One of the molecules being studied is CD15, a blood group antigen. Hence this study was done to look at the possibility of using CD15 as a prognostic marker in various thyroid neoplasms.

50 cases of thyroid neoplasms diagnosed between Jan 2006 to Sep 2009 at PSG Institute of Medical Sciences and Research, were analyzed for CD15 immuno marker expression. Thyroid neoplasms constituted about 2.16% of overall neoplasms reported during the study period. A large study by Rosai J et al showed that thyroid neoplasms accounted for 1% of the overall neoplasms and is a rare cause of death [10]. The increased incidence in our study could be due to the fact that the western part of Tamilnadu (a state in south India) which includes Nilgiris district, an endemic zone for goiter.

Out of 50 cases of thyroid neoplasms, 30 were papillary carcinomas, 15 were follicular adenomas, two cases each of medullary carcinoma and

minimally invasive follicular carcinoma and a case each of anaplastic carcinoma and follicular neoplasm of uncertain malignant potential.

Papillary carcinomas accounted for 86% of the thyroid malignancies. This incidence is significantly higher compared to western literature [46, 47] (60-80%) and also from that reported in a study from North India [10] (64%). The wide age distribution and female preponderance observed was consistent with literature.

The incidence of medullary carcinoma was 5.7% which is quite similar to the incidence observed by Rosai et al [10]. The incidence of other thyroid malignancies such as anaplastic carcinoma and minimally invasive follicular carcinoma was quite low as is observed in most studies. Most of the thyroid neoplasms were euthyroid and only 12% of the tumors showed hormonal variations. This is consistent with the observations made by several studies in the literature [46, 47].

78% of the thyroid neoplasms were < 4 cm in size. PSG Institute of Medical Sciences and Research is a tertiary care hospital and has advanced diagnostic facilities such as Ultrasound guided fine needle aspiration and Radionuclide scanning, which could have resulted in the early detection of thyroid neoplasms.

The microscopy of papillary carcinoma was of the usual type in 25 of the 30 cases while 4 were of microcarcinoma. All the 4 of the latter were less than 1 cm and were unencapsulated as is described in the literature [2, 46]. Predictably, all the 4 were incidental. 3 of them were found along with predominant areas of nodular colloid goiter and 1 was found to coexist with Hashimoto thyroiditis.

12 of the 15 follicular adenomas were of the classical type. Of the 3 variants, encountered in our study, Signet ring cell follicular adenoma posed a diagnostic challenge as metastatic deposits had to be ruled out. After extensive clinical investigation (Endoscopy, Ultrasound etc) it was labeled so. The mucin in the signet ring like cells was found to be Alcian blue positive (Fig 15) as is described in Fletchers textbook of 'Diagnostic histopathology of tumors' [9]. The histology of medullary carcinoma and anaplastic carcinoma showed no significant variations from those described in the literature.

72% of all the thyroid cancers seen in our study were either in stage I or II. This correlates with the increased incidence of small sized tumors and of papillary carcinoma in our study, apart from a female preponderance. Both the cases of medullary carcinoma were in advanced stage (IV A) which correlates with the observation of Boostrom SY et al in their article published, where they compared the staging criteria of 1997 and 2002 [48]. They argue that medullary

carcinoma may be restaged to III for tumors with N_0 status. However in our study there was no such dilemma as both the cases showed nodal involvement.

The focus of this study was to observe the incidence of CD15 expression in thyroid neoplasms as it is predicted to be a prognostic marker. CD 15 expression was analyzed in various thyroid neoplasms and the results are depicted in Table XI. Literature on CD15 expression from several studies states that these blood group antigens were not expressed in the normal thyroid tissue and that they get progressively expressed strongly in stage III papillary carcinomas and in more aggressive neoplasms. This could possibly be secondary to oncogenic transformation and subsequent increased production of Lacto-N-fucopentaose III ceramide.

As seen in Table XII and XIII, 50% of the stage III papillary carcinoma was positive for CD15 and the expression of the marker ranged from 41% to 72%. Miettinen et al and Schroder et al also observed that CD15 expression increases with increasing stage of papillary carcinoma, thus accounting for a poor prognosis. Both the cases of papillary carcinoma where we observed extrathyroidal extension showed strong CD15 positivity in more than 50% of the tumor cells. The lone case of papillary carcinoma with capsular blood vessel invasion was also positive for CD15. Thus CD15 expression was found to be high in tumors showing features which are generally indicative of a poor prognosis.

Both the cases of medullary carcinoma which were in stage IV showed high percentage of CD15 expression. This correlates with the Neuholds observation in his article published [44]. Of the 2 cases of medullary carcinoma, one showed tumor necrosis and in this case there was a very high expression (96%) of CD15. A large series of studies may be required to see the incidence of CD15 in tumors with necrosis. The lone case of anaplastic carcinoma and 2 cases of minimally invasive follicular carcinoma were negative for CD15. There is not much literature on the expression of CD15 in these cancers owing to their very low incidence.

From our study, we infer that CD15 expression was strongly expressed in papillary carcinoma of advanced stage and with poor prognostic factors like extrathyroidal extension. A similar expression was also seen in medullary carcinoma. Two of the cases of follicular adenoma with a focus of cells showing nuclear features of papillary carcinoma showed CD15 expression which might be indicative of an evolving papillary neoplasm.

SUMMARY

SUMMARY

1. Thyroid neoplasms accounted for 2.16% of the total neoplasms during the study period (3 years and 9 months).

2. Benign tumors comprised only 31% of the thyroid neoplasms, all of which were follicular adenomas,

3. 93% of the malignant thyroid neoplasms were of papillary carcinoma type.

4. Of the study group (50 cases), 15 were follicular adenoma and 35 were malignancies of which papillary carcinoma accounted for 86%.

5. CD 15 was positive in only 12 0f the 50 cases of the study group (24%).

6. 8 of the 12 CD 15 positive cases were papillary carcinoma. 2 follicular adenomas were also positive.

7. The expression of CD 15 was more common in tumors in stage III or IV and hence can be used as a prognostic marker.

8. All the malignancies (irrespective of the histologic type) which had capsular blood vessel invasion and lymph node metastasis showed CD 15 positivity.

CONCLUSION

CONCLUSION

CD 15 immunohistochemical marker is being studied as a prognostic marker for thyroid malignancies. Though only 24% of the thyroid neoplasms showed expression of CD15 in our study group, it was found to be positive in all those malignancies presenting at a higher clinical stage and also with poor prognostic indicators such as blood vessel invasion. Our study also brought out a considerable percentage of expression of CD15 in two cases of follicular adenoma, where a focus showed typical nuclear features of papillary carcinoma explaining the possibility of missing a papillary focus in an otherwise follicular adenoma. Though from our study, it is proved that CD15 can be used for prognostication and diagnosis of thyroid malignancies; a larger prospective study recruiting more number of cases is essential to prove the utility of CD 15 expression as a prognostic and diagnostic marker.

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BIBLIOGRAPHY

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MASTER CHART

S.No	HP. No	Age/ Sex	Size	Hormone status	Diagnosis	Stage	IHC %	Misc
1	150/06	30/M	2.6cm	Euthyroid	Minimally invasive follicular carcinoma	-	-	-
2	458/06	48/F	0.4cm	Euthyroid	Papillary microcarcinoma	I	-	FN-UMP (2cm)
3	747/06	49/F	3.5cm	Euthyroid	Papillary carcinoma	II	-	Multifocal, cystic
4	888/06	50/F	NA	Euthyroid	Papillary carcinoma	III	-	Extrathyroidal extension
5	1253/06	51/F	0.5cm	Hyperthyroid	Papillary microcarcinoma	I	-	Multifocal
6	1562/06	32/F	2.3cm	Euthyroid	Follicular adenoma	-	-	-
7	1801/06	38/M	1.8cm	Euthyroid	Medullary carcinoma	IV A	+	-
8	1906/06	48/M	4.2cm	NA	Follicular adenoma	-	+	-
9	2093/06	23/F	5.5cm	Euthyroid	Papillary carcinoma	III	-	-
10	2110/06	43/M	2.5cm	Euthyroid	Follicular adenoma	-	-	-
11	2230/06	57/M	3.0cm	Euthyroid	FN- UMP	-	-	-

12	2232/06	58/M	7.0cm	Euthyroid	Microfollicular adenoma- signet ring cell type	-	-	-
13	2780/06	52/F	0.4-2.3cm	Euthyroid	Papillary carcinoma	II	-	Multifocal
14	2838/06	35/F	9.5cm	Euthyroid	Follicular adenoma	-	-	-
15	3173/06	34/F	3.0cm	Euthyroid	Follicular adenoma	-	-	-
16	29/07	51/F	3cm	Euthyroid	Papillary carcinoma	II	-	Cystic
17	220/07	70/M	2.0-4.0cm	Euthyroid	Papillary carcinoma	II	+	Multifocal
18	307/07	38/M	0.5cm	Euthyroid	Papillary microcarcinoma	I	-	-
19	600/07	67/M	5.5cm	Hypothyroid	Medullary carcinoma	IV A	+	-
20	1310/07	43/F	3.3cm	Euthyroid	Follicular adenoma	-	-	-
21	1560/07	50/F	0.3-1.0cm	Euthyroid	Papillary carcinoma	I	-	Multifocal
22	1572/07	30/F	0.4cm	Euthyroid	Papillary microcarcinoma	I	-	-
23	1651/07	36/F	5.5cm	Euthyroid	Follicular adenoma	-	-	-
24	2118/07	45/F	2.5cm	Euthyroid	Follicular adenoma	-	-	-

25	2710/07	34/F	3.0cm	Euthyroid	Follicular adenoma	-	-	-
26	2874/07	65/F	1.3cm	Hypothyroid	Papillary carcinoma	I	-	-
27	3335/07	45/F	6.0cm	Euthyroid	Hurthle cell adenoma	-	-	-
28	3478/07	35/F	4.5cm	Hyperthyroid	Follicular adenoma	-	+	-
29	3679/07	27/F	3.0cm	Euthyroid	Papillary carcinoma	II	+	-
30	3693/07	46/F	0.8-5.0cm	Euthyroid	Papillary carcinoma	III	+	Multifocal
31	648/08	55/F	1.0cm	Euthyroid	Follicular adenoma	-	-	-
32	1101/08	30/F	3.0cm	Hypothyroid	Minimally invasive follicular carcinoma	-	-	-
33	1376/08	24/F	5.5cm	Euthyroid	Papillary carcinoma	III	-	Cystic
34	1422/08	53/M	2.0cm	Euthyroid	Papillary carcinoma	I	+	-
35	1837/08	30/F	0.3cm	Euthyroid	Encapsulated follicular variant of Papillary carcinoma	I	-	-
36	1865/08	29/M	2.5cm	Euthyroid	Papillary carcinoma	II	-	-
37	2062/08	35/F	4.0cm	Euthyroid	Follicular adenoma	-	-	-
38	2222/08	27/F	2.4cm	Euthyroid	Papillary carcinoma	II	-	-
39	2850/08	25/M	0.5cm	Euthyroid	Papillary microcarcinoma	I	-	-

40	3187/08	27/F	0.5cm	Hyperthyroid	Papillary microcarcinoma	I	-	-
41	4248/08	32/F	1.5cm	Euthyroid	Papillary carcinoma	I	+	-
42	11/09	45/F	3.5cm	Euthyroid	Papillary carcinoma	II	+	-
43	229/09	28/F	0.3, 1.4cm	Euthyroid	Papillary carcinoma	I	-	Multifocal, cystic
44	824/09	41/F	0.3-0.8cm	Euthyroid	Papillary carcinoma	I	+	Multifocal, stromal bone formation, FA
45	915/09	50/M	2.0cm	Euthyroid	Papillary carcinoma	III	+	Extrathyroidal extension
46	920/09	59/M	1.0-3.0cm	Euthyroid	Papillary carcinoma	II	-	Multifocal
47	1218/09	32/F	1cm	Euthyroid	Papillary carcinoma	I	-	Cystic
48	2548/09	20/F	3.5cm	Euthyroid	Papillary carcinoma	III	-	Multifocal, capsular and lymph node involvement
49	2662/09	40/F	11.5cm	Euthyroid	Anaplastic carcinoma	IVB	-	Follicular carcinoma
50	3561/09	45/F	3.5cm	NA	Papillary carcinoma	III	+	lymph node involvement, FA

CD15 IHC STAINING PATTERN IN POSITIVE CASES

PAPILLARY CARCINOMA- IHC

S.No	HP. No	AGE	SEX	SIZE	DIAGNOSIS	STAGE	*TYPE & INTENSITY*	PERCENTAGE
1	220/ 07	70	M	2.0-4.0cm	Papillary carcinoma	II	Diffuse strong membranous	100%
2	915/ 09	50	M	2.0cm	Papillary carcinoma	III	Focal strong membranous	72%
3	3561/09	45	F	3.5cm	Papillary carcinoma	III	Focal strong membranous	61%
4	11/09	45	F	3.5cm	Papillary carcinoma	II	Focal strong membranous	58%
5	3693/07	46	F	0.8-5.0cm	Papillary carcinoma	III	Focal moderately strong membranous	41%
6	4248/08	32	F	1.5 cm	Papillary carcinoma	I	Focal moderately strong membranous	28%
7	824/ 09	41	F	0.3-0.8cm	Papillary carcinoma	I	Focal strong membranous and cytoplasmic dot like	27%
8	3679/07	27	F	3.0cm	Papillary carcinoma	I	Focal moderately strong membranous	26%

MEDULLARY CARCINOMA- IHC

S.No	HP. No	AGE	SEX	SIZE	DIAGNOSIS	STAGE	*TYPE & INTENSITY*	PERCEN TAGE
1	600/ 07	67	M	5.5cm	Medullary carcinoma	IVa	Diffuse strong cytoplasmic dot like	96%
2	1801/06	38	M	1.8cm	Medullary carcinoma	IVa	Moderately strong cytoplasmic dot like	63%

Follicular adenoma- IHC

S.No	HP. No	AGE	SEX	SIZE	DIAGNOSIS	STAGE	*TYPE & INTENSITY*	PERCEN TAGE
1	1906/06	48	M	4.2 cm	Follicular adenoma	-	Focal Moderately strong cytoplasmic dot like	67%
2	3478/06	35	F	4.5cm	Follicular adenoma	-	Focal strong cytoplasmic dot like and membranous	53%